The PRIMA programme is an Art. 185 initiative supported and funded under Horizon 2020, the European Union's Framework Programme for Research and Innovation'

"Valorisation of thistle-curdled CHEESEs in MEDiterranean marginal areas" Acronym VEGGIE-MED-CHEESES

Deliverable title	**D1.3 Report 1st year**
Deliverable Lead:	Università Politecnica delle Marche
Related Work Package:	**WP1 Project coordination and overall management**
Related Task:	Task 1.3 Communication with PRIMA-IS and funding agencies
Author(s)	Lucia Aquilanti
Dissemination level	Public
Due Submission Date:	**30.04.2020 (Month 12)**
Actual submission:	**30.05.2020**
Start date of project	01.05.2019
Duration	36 months (after project end extension: 48 months)
Abstract	The 1st year Report describes the main results and goals achieved during the first year of activity of the Veggie-Med-Cheeses Consortium

Versioning and Contribution History

Version	Date	Modified by	Modification reason
V1.0	16/05/2020	Lucia Aquilanti	First version
V2.0	30/05/2020	Lucia Aquilanti	Comments after peer review process

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LIST OF PARTICIPANTS

Partner No.	Organisation	Participant (Permanent Staff)	Role
Coordinator (P1)	Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Università Politecnica delle Marche, Italy (short name: D3A-UNIVPM)	**Lucia Aquilanti**	**Project Coordinator (PC) and Principal Investigator (PI) of UNIVPM**
Partner 2 (P2)	Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - Centro di ricerca Alimenti e Nutrizione (short name: CREA-AN)	**Pamela Manzi**	**PI of CREA-AN**
Partner 3 (P3)	Departamento de Tecnologia de Alimentos y Nutrición, Universidad Católica San Antonio De Murcia (short name: UCAM)	Luis Tejada Portero	**PI of UCAM**
Partner 4 (P4)	Department of Food Hygiene and Technology, Veterinary Research Institute, Hellenic Agricultural Organization, DEMETER (short name: DEMETER)	Akis Psomas	**PI of DEMETER**
Partner 5 (P5)	High Institute of Agronomy of Chott-Mariem, SousseUniversity (Ishort name: ISA-CM)	Bouthaina Al Mohandes Dridi	**PI of ISA-CM**

1. Explanation of the work carried out by the beneficiaries and Overview of the progress

WP1 Project coordination and overall management

OBJECTIVES

The objective of WP1 was to ensure optimal co-ordination and management of VEGGIE-MED-CHEESES, as well as collaboration among Partners, with the final aim of maximising progress of knowledge and innovation outputs.

TASKS AND STATUS

Task 1.1 Organization of general Project meetings. Partners involved: PARTY 1 **(IN PROGRESS)**
Task 1.2 Project coordination, management, supervision and quality control, Partners involved: All Partners **(IN PROGRESS)**
Task 1.3 Communication with PRIMA-IS and the Funding Authorities; Partners involved: All Partners **(IN PROGRESS)**
Task 1.4 Preparation of mid-term and final reports; Partners involved: All Partners **(IN PROGRESS)**

DELIVERABLES DELIVERED IN THE REPORTING PERIOD

Deliverable number	Deliverable title	Due date of delivery	Effective date of delivery
D1.1	Kick off meeting report	30.06.2019 (month 2)	30.06.2019
D1.2	Quality Assurance Plan	31.07.2019 (month 3)	31.07.2019
D1.3	General Project meeting 2 report	30.04.2020 (month 12)	12.06.2020
D1.4	1st year (progress) report (Month 12)	31.04.2020 (month 12)	30.05.2020

BRIEF DESCRIPTION OF WORK DONE IN THE REPORTING PERIOD

- **The Coordinator organized and scheduled the Kick off meeting,** held in Italy at UNIVPM on 24th may 2019 with the participation of 4 out of 5 partners and the following agenda:

  10 h 00 – 10 h 15 **Welcome at D3A, UNIVPM and communications from the Project coordinator**

  10h 30 – 10h 45 **Flash presentation of Principal Investigators (PI), Partners, and their role in the Project**

  11h 00 - 11h 30 **Management bodies**
  • Assignation of Project Management Committee seats
  • Nomination of national expert groups
  • Drafting of the stakeholders list

  11h 30 – 11h 45 **Coffee break**

  11 h 45 – 12h 30 **First WP1 and WP7 deliverables**
  • Project Logo and type kit
  • Project Website

  13h 00-14h 30: **Lunch**

  14h 45-16h 45 **Specific techno-scientific aspects related to WP2-WP4**
  • WP2: thistle species to be sampled and collected for a preliminary screening of their milk clotting potential (sampling: who and where)
  • WP3: cultivation trials (what, who and where)

  16h 45 – 17h 00 **Coffee break**

  17h 00 – 18h 30 **Specific techno-scientific aspects related to WP5-WP6**
  • cheese-making trials and analysis of experimental and control cheeses: identification of possible pilot plants/local dairies for the manufacture of the experimental cheeses and related costs
  • consumer tests: volunteers' enrolment, protocols, details, and costs

  18h 30 **Closure of the meeting**

All the details about the meeting are reported in the Deliverable 1.1.

- The project Coordinator was ensuring the role of Project supervision with the help of the Steering Committee and the Project Management Committee. General coordination of the Project includes monitoring of the overall project progress and management of communication and knowledge transfer between participants. The quality management system was based on current practices applied at PARTY 1 institution, e.g. University and standard operating procedures applied in other EU funded projects.

- **Members of the National Expert Groups**, each comprising at least 3 members for each country, were nominated for all countries, with a Chairman who is a representative from the scientific partners and at least one expert from public bodies and one from industry. National Expert groups were acting as advisory bodies. Nominations for members of the national Expert Groups were proposed by the VEGGIE-MED-CHEESES participants; the Steering Committee evaluated and approved them. All nominated members of the national Expert Groups have a good knowledge of the national and regional sector policy and practice relevant to the sheep and goat dairy sector in their countries. Their main tasks are: (i) to be an active linkage between the Steering Committee and the main related stakeholders (academia, ministries, industries, local authorities, etc.); (ii) to have and advisory role and assist Steering Committee for the current priorities for the project implementation; (iii) to consult the Steering Committee on issues related to the evaluation of project results.
  The composition of each National Expert group is detailed as follows:

ITALY	
Prof. Raffaele Zanoli **(Chairman)**	UNIVPM (SCIENTIFIC PARTNER)
Dr Ali Bennouri	COOPERLAT FATTORIE MARCHIGIANE (Industry)
Dr Paola Staffolani	ASSAM -Agenzia Servizi Settore Agroalimentare delle Marche (PUBLIC BODY)
Dr Valeria Belelli	ASSAM -Agenzia Servizi Settore Agroalimentare delle Marche (PUBLIC BODY)
GREECE	
Dr. George Samouris **(Chairman)**	DEMETER, Research Director (SCIENTIFIC PARTNER)
Mrs Tania Tatsika	Food Scientist, MSc, Hellenic Food Safety Authority (PUBLIC BODY)
Mr Dimitris Minopoulos	Animal Production technologist, owner of "Amalthea" Farm (INDUSTRY)
SPAIN	
Prof. Eva Salazar **(Chairman)**	UCAM, Research Team Member (SCIENTIFIC PARTNER)
Mr Javier Muñoz Pérez	Technical Director. P.D.O. Torta del Casar (INDUSTRY)
Mr Adolfo Falagan Prieto	President of Consejo Regulador de las Denominaciones de Origen Protegidas "Queso de Murcia" y "Queso de Murcia al Vino" (INDUSTRY)
Prof. Rafael Gómez Díaz	Universidad de Córdoba. Food Technology Area Research group AAGR-120 Lactology and Meat Technology (PUBLIC BODY)
TUNISIA	
Prof. Bouthaina Al Mohandes	ISA-CM, Research Team Member (SCIENTIFIC PARTNER)

(Chairman)	
Dr Hanen Ben Ismail	Associate Professor in agrifoods industries Mr Adolfo Falagan (PUBLIC BODY)
Mr Riadh Louhichi	Director of Organization of Sectors and Promotion of the Quality of Animal Products at the Interprofessional Group of Red Meats and Milk "GIVLAIT" Tunisia (PUBLIC BODY)

- **The Coordinator organized and scheduled the 1st year General meeting,** held via teleconference (using TEAMS platform) due to the COVID-19 pandemic emergence, on 8th June 2020 with the participation of all the partners and the following agenda:

  10 h 00 – 10 h 15 (Rome time) **Connection and Greetings**

  10h 15– 11h 15 (Rome time) **Presentation of the main results achieved by Research partners involved in WP 2 and 3**
  11h 00 - 12h 30 (Rome time) **Presentation of the main results achieved by Research partners involved in WP 4**

  12h 30 – 13h 15 (Rome time) **Discussion and issues from the Partners**
  13h 30 (Rome time) **Closure of the meeting**
  12h 30 – 13h 15 **Discussion and issues from the Partners**

  All the details about the meeting are reported in the Deliverable 1.1.

- The Coordinator reviewed the **periodic reports** prepared by the WP leaders to verify their consistency with the project tasks, in view of their assembling and transmitting to PRIMA-IS and the funding agencies on behalf of the Consortium.

- The Coordinator drafted the 1st year Report

WP2 Characterization of spontaneously grown thistle populations

OBJECTIVES

The objective of WP2 is the characterization of Mediterranean spontaneous thistle populations ascribed to species and genera traditionally exploited in local cheese-making for the manufacture of thistle-curdled sheep's and goat's milk cheeses: C. *humilis, C. acanthifolia* and *O. tauricum*. To reach this goal both qualitative and quantitative morphological traits are evaluated, to eventually identiy different ecotypes (even using genetic markers).

TASKS AND STATUS

Task 2.1 Sampling and morphological characterization of spontaneously growing thistles; Partners involved: PARTY 1, PARTY 5 **(IN PROGRESS)**
Task 2.2 Lyophilization of sampled spontaneous thistles; Partners involved: PARTY 1, PARTY 5 **(COMPLETED)**
Task 2.3 Sampling, cataloguing, and storing of seeds; Partners involved: PARTY 1, PARTY 5 **(COMPLETED)**

Task 2.4 Elaboration of morphological data and potential identification of different ecotypes; Partners involved: PARTY 1, PARTY 5 **(IN PROGRESS)**

DELIVERABLES DELIVERED IN THE REPORTING PERIOD

Deliverable number	Deliverable title	Due date of delivery	Effective date of delivery
D2.1	Lyophilized biomass from spontaneous thistle populations	31.07.2019 (month 3)	30.07.2019
D2.2	Catalogued and stored seeds from Mediterranean spontaneous thistle populations	01.01.2020 (month 19)	31.10.2019

BRIEF DESCRIPTION OF WORK DONE IN THE REPORTING PERIOD

Morphological characterization is the first step in the description and classification of germplasm. Accordingly, the genetic diversity in spontaneous thistles populations ascribed to *Cynara humilis*, *Onopordum platylepis* and *Onopordum tauricum*, occurring in different Mediterranean marginal areas (high altitude pastures, dry and arid lands, wasteland, etc.) were morphologically characterized based on qualitative and quantitative traits, thus leading to the identification of different geographical populations and even ecotypes. Samples and seeds from these populations were collected, catalogued, and stored. Sampling was performed within the natural areas of distribution of the thistle species under study, spanning from Spain in the West Mediterranean passing through North Africa and Italy.

The WP2 activities carried out in the reporting period are briefly summarized as follows:

- Samples from thistle populations spontaneously occurring in various Mediterranean marginal areas (PARTY 1: central Italy; PARTY 5: South-eastern Spain, North Tunisia), ascribed to the species *Cynara humilis*, *Onopordum tauricum* and *Onopordum platylepis* have been collected, classified, and characterized through the evaluation of morphological (qualitative and quantitative) traits, according to the UPOV (International Union for the Protection of New Varieties of Plant) for artichoke. Sampling has been carried out using the simple random method and a specimen of each population has been catalogued and stored with notes of collecting areas. Sampling was performed according to the following scheme:
  - *Onopordum tauricum* has been sampled from two Italian sites (Cupi di Visso, Macerata; Colfiorito, Perugia)
  - *Onopordum platylepis* has been sampled from one Tunisian site (Chott Meriem in the central-eastern regions of Tunisia)
  - *Cynara humilis* has been sampled from one Spanish site (Alcaraz, Castiglia-La Mancia)

  More details about sampling are reported in D2.2.

- Sampled thistles have been lyophilized (as capula from thistle flours) according to a standard procedure detailed in D2.1. The dried material has been crushed and the resulting powder has been kept in sealed bags at room temperature for storing.

- Seeds have been catalogued according to international protocols (ISTA, 2018), and further stored in rooms at controlled temperature (15°C) and relative humidity (15%) and in freezer cabinets at -20°C; all details about seeds cataloguing and storage are reported in D 2.2.
- Morphological data are being now elaborated using multiple statistical tools (PCA, PLS, PLS-DA, UPGMA cluster analysis, etc.) to identify eventual different thistle ecotypes (PARTY 1). For *Onopordum tauricum*, two ecotypes within the two sampled populations have been identified based on morphological traits; molecular tests based on the analysis of Simple Sequence Repeats (SSR) markers are currently in progress for the evaluation of the genetic variability between these two ecotypes.

WP3 Sustainable cultivation of thistles

OBJECTIVES

The objective of WP3 is the evaluation of the adaptability and agronomic productivity of *C. humilis*, *O. tauricum* and *Onopordum platylepis* in rainfed areas of the Mediterranean basin under zero/low inputs of fertilizers and use of non-chemical weed control methods. The final goal of this WP is to produce recommendations and guidelines for the sustainable cultivation, in the Mediterranean basin, of thistles exploitable by dairy industries for the manufacturing of high-quality and safe thistle-curdled cheeses.

TASKS AND STATUS

Task 3.1 Design of the field experiments; Partners involved: PARTY 1, PARTY 5 **(COMPLETED)**
Task 3.2 Germination tests; Partners involved: PARTY 1, PARTY 5 **(COMPLETED)**
Task 3.3 Soil characterization; Partners involved: PARTY 1, PARTY 5 **(COMPLETED)**
Task 3.4 Seeds multiplication and transplantation; Partners involved: PARTY 1, PARTY 5 **(IN PROGRESS)**
Task 3.5 Phenological development survey; Partners involved: PARTY 1, PARTY 5 **(IN PROGRESS)**
Task 3.6 Analysis of the above ground fresh and dry biomass; Partners involved: PARTY 1, PARTY 5 **(NOT STARTED)**
Task 3.7 Lyophilization of sampled cultivated thistles; Partners involved: PARTY 1, PARTY 5 **(NOT STARTED)**

DELIVERABLES DELIVERED IN THE REPORTING PERIOD

Deliverable number	Deliverable title	Date of delivery	Effective date of delivery
D3.1	Experimental plan design of cultivation trials	31.07.2019 (month 3)	31.07.2019

BRIEF DESCRIPTION OF WORK DONE IN THE REPORTING PERIOD

Field experiments have been planned in 2 experimental sites, each modelling a Mediterranean scenario with different landscapes and climatic conditions, namely:

- a central Italian hill area next to the Adriatic coast, with a warm and rainy temperate climate (classified as Csa = hot-summer Mediterranean climate, according to Köppen and Geiger) at least three times as much precipitation in the wettest month of winter as in the driest month of summer and driest month of summer receives less than 30 mm (1.2 in) (PARTY 1);

- an area in North Tunisia with an arid climate (classified as BSh = hot semi-arid climate, according to Köppen and Geiger), which is characterized by scarce rainfall and high temperatures during the whole year (PARTY 5).

In both the two sites the following species and ecotypes have been compared: *Cynara humilis* L., *Onopordum tauricum* Willd (two ecotypes),*Onopordum platylepis* Murb., *Cynara cardunculus* L. This latter species has also been included, thought its cultivation was not foreseen in the original Workplan (see for more details section 5).

The WP3 activities carried out in the reporting period are briefly summarized as follows:

- Selected thistle species/ecotypes have been compared in a randomized block experimental design with three replications. **Design of the experimental plan at the two selected sites is detailed in** D3.1.
- Seeds have been subjected to germination tests to identify the most suitable environmental conditions for their germination (temperature, light, pre-treatments, etc.).
- Influence of soil composition on thistles-based cropping system has been evaluated by measuring key soil parameters with standardised procedures already in use at the laboratories of the PARTY 1 and PARTY 5, being pH, organic matter content, electrical conductivity, total N, exchangeable K2O, available P2O5, exchangeable Ca, Na and Mg.
- Regarding the sole Italian site, in March 2020 seeds have been planted in standard seed trays; four-week-old thistles with three-four true leaves have been transplanted (late April 2020) on previously prepared plots. Supplementation of low doses of organic fertilizer and relatively spaced irrigations (depending on rainfall). Tillage consisted of medium-depth ploughing (30 cm). Seed bed preparation has been conducted immediately before planting, by using a disk harrow. Weed are regularly eliminated by using non-chemical methods (hoeing).
- Regarding the Tunisian site, seed germination and young plants transplantation will be carried out on September 2020.
- At the Italian site, the monitoring of plants survival has been started in late April 2020 and it will be performed twice per growing season, as foreseen in Task 3.5. In the first monitoring at the beginning of the plant cycle, the number of plants that has re-grown will be recorded. In the second monitoring at the end of the season, the number of plants that has reached reproduction will be recorded. Plant survival will be then estimated as the ratio between the second and first monitoring. In parallel, twenty plants have been randomly selected within each plot and phenological growth stages are regularly recorded (every 3 days), according to the universal BBCH coding system for mono-and dicotyledonous plants (BBCH abbreviation stands for Biologische Bundesanstalt, Bundessortenamt and Chemical industry). Environmental parameters (rainfall and daily maximum and minimum air temperature) are regularly recorded at an agro-meteorological station located in of the two experimental field.

WP4 Characterization of thistle aqueous crude extracts (CEs)

OBJECTIVES

The objective of WP4 is the full characterization (chemical, microbiological, technological and biochemical) of aqueous crude extracts (CEs) derived from both spontaneously grown and cultivated thistles (CE_st and CE_ct, respectively) from either whole plants or separate plant sections: stalks, leaves, capitula.

TASKS AND STATUS

Task 4.1 Chemical characterization of CEs; Partners involved: PARTY 1, PARTY 2, PARTY 5 **(IN PROGRESS)**
Task 4.2 Microbiological characterization of CEs; Partners involved: PARTY 1, PARTY 3, PARTY 4, PARTY 5 **(IN PROGRESS)**
Task 4.3 Evaluation of technological properties of CEs; Partners involved: PARTY 1, PARTY 3, PARTY 4 **(IN PROGRESS)**
Task 4.4 Purification of proteases from CEs; Partners involved: PARTY 1 **(IN PROGRESS)**
Task 4.5 Biochemical characterization of purified proteases; Partners involved: PARTY 1 **(IN PROGRESS)**

DELIVERABLES DELIVERED IN THE REPORTING PERIOD

None

BRIEF DESCRIPTION OF WORK DONE IN THE REPORTING PERIOD

- In this WP, fresh crude extracts (CEs) prepared by reconstituting the lyophilized biomass of sampled thistles (*Onopordum tauricum*, *Onopordum platylepis*, *Cynara humilis*) from spontaneously grown Mediterranean populations (CE_st) have been analysed for the following chemical parameters (using standardised procedures already in use at the laboratories of the Partners):
    - hydrosoluble phenolics (PARTY 1);
    - minerals (Ca, P, Na, K, Mg, Zn) by atomic absorption spectroscopy (AAS) (PARTY 2);
    - B-group vitamins by High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD) and High-Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) (PARTY 1);
    - antioxidant properties (PARTY 1).
- CE_st were also subjected to viable plate counting of quality and hygiene indicators (total mesophilic aerobes, Enterobacteriaceae and *Escherichia coli*, eumycetes, spore-forming bacteria, pseudomonads) and pro-technological microorganisms (mesophilic and thermophilic lactic acid bacteria) (PARTY 4) on selective/semi-selective solid media and under opportune growth conditions; the antibacterial activity of CEs has also been assessed at PARTY 1 laboratories.
- Milk-clotting activity (MCA) of CEs was measured according to the International Dairy Federation Standard. Proteolytic activity (PA) has also been assessed on caseins from sheep, goat and bovine milk using photometric assays, as previously described by **Tavaria et al. (2001**). Protein concentration has been determined with the Bradford method (**Bradford, 1976**) using bovine-serum albumin as reference standard for the calibration curve. MCA and PA have been evaluated under different conditions (e.g. pH, T). Finally, Response Surface Methodology (RSM) was used to identify optimal conditions for milk clotting.
- CE_st from *Onopordum tauricum*, characterized by the best performance in terms of milk clotting ability, was chosen to start the purification and further characterization of proteases to be potentially exploited by the dairy industry for the manufacturing of thistle-curdled cheeses.
- Different steps have been carried out to purify the active proteolytic fractions from *O. tauricum*, including salting-out and ion exchange chromatography, according to Llorente et al, 2004.
- The biochemists working at D3A (coordinator) have just started the biochemical characterization of the purified protease from *Onopordum tauricum*, according to the activities detailed in the Task 4.5.

WP5 Cheese-making trials and characterization of thistle-curdled and control cheeses

OBJECTIVES

The objective of WP5 is to carry out cheese-making trials allowing for the full characterization (physico-chemical, chemical, microbiological, textural and sensory) of local thistle-curdled cheeses as well as for the investigation of nutritionally valuable substances (e.g. minerals, vitamins, etc), health-beneficial (e.g. phenolic compounds, bioactive peptides with ANTI-ACE activity) and hazardous (biogenic amines) compounds

TASKS AND STATUS

Task 5.1 Cheese-making trials; Partners involved: PARTY 1, PARTY 3, PARTY 4 (NOT STARTED)
Task 5.2 Physico-chemical and chemical analyses; Partners involved: PARTY 1, PARTY 3, PARTY 4 (NOT STARTED)
Task 5.3 Microbiological analyses; Partners involved: PARTY 1, PARTY 4 (NOT STARTED)
Task 5.4 Textural and sensory analyses; Partners involved: PARTY 2, PARTY 3, PARTY 4 (NOT STARTED)
Task 5.5 Analysis of nutritionally valuable, health-beneficial, and hazardous substances (Month 8-Month 14: cheeses made with CE_st; Month 22-Month 28: cheeses made with CE_ct); Partners involved: PARTY 1, PARTY 2, PARTY 3 (NOT STARTED)
Task 5.6 Statistical elaboration of data; Partners involved: PARTY 1 (NOT STARTED)

DELIVERABLES DELIVERED IN THE REPORTING PERIOD

None

BRIEF DESCRIPTION OF WORK DONE IN THE REPORTING PERIOD

Not applicable

WP6 Evaluation of consumer needs, preferences, and acceptance towards thistle-curdled and control

OBJECTIVES

The objective of WP6 is to evaluate the degree of liking of consumers towards experimental and control "second round" cheeses, as well as to provide analytical insights on the prospect value-chain potential of thistle-curdled traditional local cheeses in the Mediterranean

PLANNED TASKS AND STATUS

Task 6.1 Focus groups and consumer tests; Partners involved: PARTY 1, PARTY 3, PARTY 4 (NOT STARTED)
Task 6.2 Prospect value chain analysis and business model canvas analysis; Partners involved: PARTY 1, PARTY 3, PARTY 4 (NOT STARTED)

DELIVERABLES DELIVERED IN THE REPORTING PERIOD

None

BRIEF DESCRIPTION OF WORK DONE IN THE REPORTING PERIOD

Not applicable

WP7 Multi-actor internal and external communication and technology transfer

OBJECTIVES

The objectives of WP7 are to ensure participation of stakeholders, as well as dissemination and exploitation of VEGGIE-MED-CHEESES outputs. A distinction can be made between objectives of internal and external communication.

Objectives of **internal communication** (within the Project), are:

I. communicate project results within the Consortium so that all partners are updated timely on the knowledge generated by all WPs and tasks;
II. coordinate the use of participatory approaches to utilise stakeholder and research knowledge and innovation to prioritise research activities within the project.

Objectives of **external communication** (to Stakeholders), are:

I. use participatory approaches to utilise stakeholder and research knowledge to prioritise dissemination and training activities;
II. communicate and disseminate project outputs to stakeholders and create a dairy farm-level observatory and knowledge exchange network;
III. build capacity through technology transfer to ensure that the industry can effectively use the outputs from the project.
IV. create a roadmap for future implementation and exploitation of project outputs at dairy farm, on regional and Mediterranean scales and for further research.

As a whole these actions aim to promote participation and effectively translate the Project outputs to meet the needs of the dairy industry and so that the results are disseminated in a way that can be promptly implemented and exploited by the following stakeholder groups: i) crop producers; ii) dairy farms, dairy industries, dairy operators, iii) retail and consumers and the general public; iv) Non Governative Organisations; v) scientists; vi) policy makers. All the partners will be involved in this WP.

TASKS AND STATUS

Task 7.1 Establishment of a stakeholder-platform (Month 1–Month 36); Partners involved: All partners **(IN PROGRESS)**
Task 7.2 Installation of a website and production of dissemination materials; Partners involved: All partners **(IN PROGRESS)**
Task 7.3 Documentation of scientific results; Partners involved: All partners **(IN PROGRESS)**

DELIVERABLES DELIVERED IN THE REPORTING PERIOD

Deliverable number	Deliverable title	Due date of delivery	Effective date of delivery
D7.1	Project logo, fonts and communication templates	31.05.2019 (month 1)	31.05.2019
D7.2	Public VEGGIE-MED-CHEESES website	30.06.2019 (month 2)	30.06.2019
D7.3	VEGGIE-MED-CHEESES intranet	30.06.2019 (month 2)	30.06.2019
D7.4	Press release at Project launch for the wide public	31.07.2019 (month 3)	31.01.2020
D7.5	Establishment of a stakeholder-platform as a tool for industry level observatory and knowledge exchange network	31.10.2019 (month 6)	31.10.2019
D7.6	VEGGIE-MED-CHEESES Data management plan	31.10.2019 (month 6)	31.10.2019

BRIEF DESCRIPTION OF WORK DONE IN THE REPORTING PERIOD

- As detailed in the Communication, Dissemination and Exploitation Plan described in section 2.2, different tools were adopted to ensure that the outputs of the Project are communicated to the public, the relevant stakeholders, etc., including: I. Project website; II: Project leaflet; III. newsletters; IV. social media; V. articles in farmers/dairy industries journals. At this regard, a visual identity defining the Project's graphic character, logo, fonts, colours have been designed including compulsory logos that must be used by all Partners for communication about Project activities. For details on Project logo, fonts and communication templates see D7.1.

- A Project website for external and internal communication, dissemination and project management was established by the PARTY 1 at a suitable server to: I. Present information on Project structure and outputs; II. Allow closed and open discussion between Partners and Stakeholders; III. Act as the Project website for VEGGIE-MED-CHEESES. The website has an open site and a restricted site. The restricted site has a state-of-art technology with password-regulated access for Project Partners and PRIMA Secretariat to have access to internal and confidential material with a special section for the stakeholder platform. The restricted site contains: I. Updated Projects documents including all meeting agendas, material, and reports at overall Project level and by WPs; II: Project reports; III. Facility to share Project data to facilitate exchange between Partners; IV. Share point to enable information exchange and discussion between Partners; V. Share point to allow discussion between Partners and the stakeholder platform. The open site contains: I. Project objectives, list of Partners (including contact details and web site links) and Project description; II. Press releases and latest news; III. Calendar and information on Project events; IV. Scientific publications, presentations from conferences and proceedings; V. Regular update on results-outputs of the Project including recommendations, guidelines, technical notes, and summary articles from the Project activities. For more details on the public and private parts of the Project website see D7.2 and D7.3, respectively.

- A stakeholder platform, composed of up to 3 members per participating country, was established to represent the interests of the target- and user -groups. The Consortium Partners identified the key stakeholders for each participating country, ensuring gender and group balance and promoting inclusion

of crop producers, small dairy farms/dairy operators, etc. Selected stakeholders periodically received updated information/news about the project and its progresses and main outcomes (4 newsletters have already been sent, since the start of the project) (for details on Stakeholder platform see D7.5)

- A data management plan was drafted and completed by Month 6, to manage the VEGGIE-MED-CHEESES data overall collected from stakeholders as well as qualitative and quantitative analyses foreseen in WP2, 3, 4, 5, and 6. The data management plan was drafted according to the H2020 Guidelines on Data management, including issues such as: "What types of data will the project generate/collect? What standards will be used? How will this data be exploited and/or shared/made accessible for verification and re-use? How will this data be curated and preserved?" This plan will be annually discussed and updated (see for detailed D7.6)

2. Update of the plan for exploitation and dissemination of result

The plan for exploitation and dissemination of results as described in the proposal submitted was not updated.

3. Update of the data management plan

A new Data Management Plan (DMP) was drafted (Deliverable 7.6). The aim of the VEGGIE-MED-CHEESES' DMP is to identify the project's research data and to describe how to make them findable, accessible, interoperable and re-usable (FAIR). Following the H2020Data Management Plan Template, all partners involved in research's activities were asked to provide detailed information about the data generated during the entire project. In more detail, in the Deliverable, an initial analysis on how the VEGGIE-MED-CHEESES Consortium intends to manage the amount of data produced in the VEGGIE-MED-CHEESES project is reported. The first checkpoint of the whole architecture of the DMP is the release of the first scientific publication that will be published within the VEGGIE-MED-CHEESES project: indeed, the data reported in this paper will be made available and interoperable to the larger typologies of stakeholders. To avoid issues related to Principal Investigators (PI) rights and their access, as a first step in the strategy of development of DMP, only data related to publications available to the public will be released. In the VEGGIE-MED-CHEESES Project DMP is intended to be a living document in which information can be made available on a finer level of accuracy and details through updates as the implementation of the project progresses and when significant changes occur. All details related to DMP are given in D 7.6.

4. Follow-up of recommendations and comments from previous review(s)

Not applicable

5. Deviations from original proposal

A few deviations from the original proposal have been foreseen; they are described as follows:

- **REPLACEMENT OF CARLINA ACANTHIFOLIA WITH ONOPORDUM NERVOSUM SSP. PLATYLEPIS (TASK 2.1 - 2.4; 3.2; 3.4 - 3.7; 4.1 - 4.5; 5.1 – 5.6; 6.1 – 6.2)**

A new thistle species – ***Onopordum nervosum subsp. platylepis*** (Murb.) has been considered for: morphological characterization, cultivation and analysis of the CE prepared by maceration in cold tap water of its flowers. This species has selected to replace the species *Carlina acanthifolia* supsp. *acanthifolia*, since according to preliminary tests performed by biochemists working at the D3A-UNIVPM laboratories, *C. acanthifolia* does not contain any proteinaceous milk-clotting agent. *Onopordum platylepis* belong to the family *Asteraceae*; it grows spontaneously in Algeria, Libia and Tunisia, being hence included in the African Plant Database (http://www.ville-ge.ch/musinfo/bd/cjb/africa/details.php?langue=an&id=163821). It is a thorny bisanual plant, robust, very tomentose, with erect stem, little branching with broad thorny wings, from 0.50 to 1.50 m. Flowering occurs in May – June, whereas dealing with its ecology it grows along roadsides and in uncultivated places. In Tunisia, women from small villages are known to use flowers from this species for family-run manufacturing of raw milk cheeses. Accordingly, given the acknowledged skills and experience gained by botanists working at ISA-CM, flowers and seeds from this species have been collected in June-July 2019. The seeds have been catalogued, stored, and an aliquot sent to UNIVPM for the cultivation trials, whereas flowers have been used for the preparation of the crude extract (CE); this latter has been hence lyophilized and sent to UNIVPM, DEMETER, CREA-AN and UCAM for its full characterization.

- **SUBCONTRACTING TO MAJELLA SEED BANK – MAJELLA NATIONAL PARK (ITALY) https://www.parcomajella.it/en/**

  An unforeseen subcontracting to Majella Seed Bank (Italy) has been used for sampling of flowers and seeds of *Onopordum tauricum* from the Abruzzo and Marche Region (Central Italy) as well as for optimization of germination of seeds from this species.

  The Majella Seed Bank deals with the *ex situ* conservation of rare and/or endangered, endemic species suitable for renaturalization in vulnerable or damaged ecosystems within the Park. Mjella Seed bank is a founding member of the Italian Network of Germplasm Banks for the ex situ conservation of Italian flora (RIBES), whose function is to constitute a reserve of seeds of wild plants at greatest risk of extinction, thus implementing a strategy for nature conservation complementary to the in situ conservation strategies ensured by parks and reserves, in line with the objectives of the international conventions on nature protection.

  The various plant entities are subject to a specific collection protocol, as specified in the guidelines of the conservation program of the "Millenium Seed Bank (MSBP)", conceived and developed by the Royal Botanic Gardens (Kew Garden, UK). At each accession they are entered in a database to allow their identification with a reference code. The samples collected in the field are checked, verified, and subjected to a series of qualitative and quantitative tests (viability and germinability). Subsequently the seeds are treated inside the dehydration chamber, in which the percentage of humidity is lowered; at the end of the dehydration phase, the sample is packaged and placed in the freezer at a temperature of -18 ° C, to guarantee long-term conservation. The structure is located inside the Michele Tenore Botanical Garden of Lama dei Peligni, and hosts students and researchers who carry out thesis and research activities promoted by the Park Authority.

- **DIRECT PRAPARATION OF CRUDE EXTRACTS (CE_st) FROM TUBULAR FLOWERS AND LYOPHILIZATION OF THE RESULTING EXTRACTS IN PLACE OF LYOPHILIZATION OF WHOLE THISTLE PLANTS AND PLANT SECTIONS (LEAVES, STEMS, FLOURS) (TASK 2.1)**

TASK 2.1 "Lyophilization of sampled spontaneous thistles" was slightly modified as follows. Based on preliminary tests carried out at UCAM and UNIVPM laboratories, the Consortium partners agreed in sampling (and hence analysing) flowers heads collected from the three thistle species considered. Hence, tubular flowers were manually separated from receptacles, immediately after harvesting and macerated in cold tap water for production of crude extracts (CE_st); the latter were immediately subjected to freeze-drying, according to a standardized process.

- **EXCLUSIVE ANALYSIS OF CRUDE EXTRACTS PREPARED FROM TUBULAR FLOWERS COLLECTED FROM THISTLE CAPITOLA (CE_ST) (WP4, ALL TASKS)**

  As far as WP4 activities are considered, crude extracts prepared by maceration of tubular flowers collected from capitola sampled from spontaneous thistles (CE_st) were exclusively analysed. The decision of not preparing and hence analysing crude extracts from macerated thistle leaves or stems was based on the preliminary results collected at UNIVPM and UCAM demonstrating the very low concentration of milk-coagulating agents in these thistle plant sections.

- **INCLUSION OF CYNARA CARDUNCULUS IN THE CULTIVATION TRIALS AT BOTH THE ITALIAN AND TUNISIAN SITE (TASK 3.1, 3.2, 3.4 – 3.6)**

Based on a proposal of a Consortium Partner (Prof. Bouthaina Dridi Al Mohandes, ISA-CM) and the specific interest of some project stakeholders (Spanish dairy farm producing thistle-curdled cheeses), the species *Cynara cardunculus* has been included in the cultivation trials at both the Italian and Tunisian sites to optimize its cultivation and collect data to draft specific guidelines for its cultivation in the Mediterranean basin. The cultivation of this species was not foreseen in the original plan and represents and additional activity, thus increasing the value of the overall proposal. The choice to ample the number of thistle species to be tested in open field experiments respond to a specific request of the Mediterannean dairy industries already exploiting vegetable coagulants, complaining about the poor commercial availability of coagulants from this well-characterized species. Undoubtedly a more widespread and/or optimized cultivation of *C. cardunclus* might support the increasing demand for a vegetable coagulant produced from this specific species.

- **SLIGHT VARIATION IN THE MICROBIOLOGICAL ANALYSES OF CRUDE EXTRACTS FROM SPONTANEOUS THISTLES (CE_st) (TASK 4.1)**

S. aureus was not included among human pathogens used as indicator microorganisms for the assessment of the antimicrobial activity of the aqueous crude extracts prepared from spontaneous thistles; the surrogate microorganism *Listeria innocua* was used in place of *L monocytogenes*